

Submission ID #: 69529

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Project Page Link: <https://review.jove.com/account/file-uploader?src=21206393>

Title: In Vitro Reassociation Assay to Measure the Formation of 80S Ribosomal Particles Using Salt-Washed Ribosomal Subunits

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Author Questionnaire

1. We have marked your project as author-provided footage, meaning you film the video yourself and provide JoVE with the footage to edit. JoVE will not send the videographer. Please confirm that this is correct.

✓ Correct

2. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

3. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

4. Proposed filming date: To help JoVE process and publish your video in a timely manner, please indicate the proposed date that your group will film here: **02/12/2026**

When you are ready to submit your video files, please contact our Content Manager, [Utkarsh Khare](#).

Current Protocol Length

Number of Steps: 10

Number of Shots: 23

Introduction

INTRODUCTION:

~~What is the scope of your research? What questions are you trying to answer?~~

- 1.1. **Analiis Veeremaa**: Our research investigates the association of ribosomal subunits during translation initiation, elucidating how interactions between subunits regulate efficient protein synthesis in cells universally

- 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~What research gap are you addressing with your protocol?~~

- 1.2. **Anette Hallik**: This protocol addresses the lack of methods to analyze ribosomal subunit joining independent of active translation.

- 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

~~What advantage does your protocol offer compared to other techniques?~~

- 1.3. **Anette Hallik**: The advantage of this protocol compared to other techniques is the ability to test reassociation of ribosomal subunits under controlled magnesium concentrations.

- 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~What are the current experimental challenges?~~

- 1.4. **Analiis Veeremaa**: The current experimental challenges involve limitations of existing methods that prevent direct analysis of ribosomal subunit joining during translation.

- 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~What new scientific questions have your results paved the way for?~~

- 1.5. **Anette Hallik**: The new scientific questions paved by these results involve how ribosomal proteins, RNA, and ligands specifically regulate ribosomal subunit joining.
 - 1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Protocol

2. Preparation and Fractionation of Yeast Ribosomal Complexes Using Linear Sucrose Density Gradients

Demonstrator: Anette Hallik

- 2.1. To begin, prepare six 36-milliliter linear sucrose gradients with Buffer A in polypropylene centrifuge tubes using a gradient mixer [1-TXT]. Add 18 milliliters of 30% sucrose solution to the gradient mixer well positioned further from the exit tubing [2].
 - 2.1.1. WIDE: Talent setting up the gradient mixer with polypropylene centrifuge tubes. **TXT: Gradient: 10 - 30 %**
 - 2.1.2. Talent adding 18 milliliters of 30 percent sucrose solution into the gradient mixer well farther from the exit tubing.
- 2.2. Then add 20 milliliters of 10% sucrose solution to the well next to the exit tubing [1]. Ensure that no air bubbles enter the gradient during mixing [2].
 - 2.2.1. Talent adding 20 milliliters of 10 percent sucrose solution into the gradient mixer well next to the exit tubing.
 - 2.2.2. Close-up shot showing smooth gradient formation without air bubbles.
- 2.3. Carefully pipette out some of the solution from the surface of each gradient to balance the gradients [1]. Then load up to 150 units of the yeast ribosomal lysate onto the top of each sucrose gradient [2].
 - 2.3.1. Talent adjusting gradient volumes by removing small amounts of solution from the top.
 - 2.3.2. Talent carefully loading lysate onto the surface of the sucrose gradient.
- 2.4. Now place the gradients into a swinging-bucket rotor [1] and ultracentrifuge at 50,339 g for 16 hours at 4 degrees Celsius [2].
 - 2.4.1. Talent loading centrifuge tubes into the swinging-bucket rotor in the ultracentrifuge.
 - 2.4.2. Talent setting centrifuge parameters.
- 2.5. Set up the gradient fractionator or a similar system containing a glass capillary tube, a peristaltic pump, a spectrophotometer, a fraction collector, and a writer [1].
 - 2.5.1. Talent assembling the gradient fractionation setup on the laboratory bench.

- 2.6. Measure the time required for an air bubble to travel from entering the spectrophotometer to exiting the output tubing [1].
 - 2.6.1. Talent introducing an air bubble into the system to determine lag time.
- 2.7. Place the centrifuge tube on ice [1]. From the top of the tube, slide the glass capillary tube straight to the bottom of the gradient [2]. Then pump the gradient through the setup from the bottom to the top while measuring absorbance at 260 nanometers [3].
 - 2.7.1. Talent placing the centrifuge tube on ice.
 - 2.7.2. Talent inserting the glass capillary tube vertically to the bottom of the gradient.
 - 2.7.3. Shot of the Spectrophotometer display showing absorbance at 260 nanometers during gradient pumping.
- 2.8. When the 80S peak appears on the recorded graph, set a timer for this lag time [1], and begin collecting the gradient only after the lag time has passed [2-TXT].
 - 2.8.1. Shot of the 80S peak on the recorded graph.
 - 2.8.2. Talent starting fraction collection after the defined lag time. **TXT: Use same lag time to stop collection**

3. Reassociation of Eukaryotic Ribosomal Subunits

Demonstrator: Analiis Veeremaa

- 3.1. Prepare six 11 milliliter linear sucrose gradients with Buffer R in polypropylene centrifuge tubes using the gradient mixer [1]. Add 5.5 milliliters of 30% sucrose solution to the gradient mixer well positioned further from the exit tubing [2] and add 7 milliliters of 10 percent sucrose solution to the well next to the exit tubing [3-TXT].
 - 3.1.1. WIDE: Talent preparing smaller polypropylene centrifuge tubes in the gradient mixer.
 - 3.1.2. Talent adding 5.5 milliliters of 30 percent sucrose solution into the appropriate mixer well.
 - 3.1.3. Talent adding 7.0 milliliters of 10 percent sucrose solution into the adjacent mixer well. **TXT: Ensure no bubbles enter the gradient**
- 3.2. Balance the prepared sucrose gradients pairwise by carefully removing solution from the surface of each gradient [1]. Then load 300 microliters of the *in vitro* reassociation reaction carefully onto the top of sucrose gradient [2].
 - 3.2.1. Talent equalizing gradient volumes by removing excess solution from the top.
 - 3.2.2. Talent gently loading the reaction mixture onto the gradient surface.
- 3.3. Place the gradients into a swinging-bucket rotor [1] and ultracentrifuge at 37,368 g for 20 hours at 4 degrees Celsius [2].
 - 3.3.1. Talent positioning tubes into the swinging-bucket rotor.

3.3.2. Talent placing the rotor into the ultracentrifuge and initiating the run.

3.4. Using the gradient fractionation system, pump the gradient from the bottom to the top while measuring absorbance at 260 nanometers [1]. Record the absorbance of one tube at a time with the spectrophotometer writer [2].

3.4.1. Talent recording a graph of absorbance.

3.4.2. Talent recording individual absorbance profile for one tube

Results

4. Results

Video Editor: For all graphs, please add the grey label showing the 10% to 30% sucrose (at the bottom)

4.1. Centrifugation of the clarified lysate on a 10% to 30% sucrose gradient revealed a single peak corresponding to 80S ribosomal particles in the bottom two-thirds of the gradient [1], along with a peak of low molecular weight nucleic acids near the top [2].

4.1.1. LAB MEDIA: Figure 2A. *Video editor: Highlight the large peak labeled "80S".*

4.1.2. LAB MEDIA: Figure 2A. *Video editor: Highlight the peak at the top of the gradient, near the 10% sucrose end.*

4.2. After high-salt dissociation of purified 80S particles, centrifugation revealed two peaks corresponding to 40S and 60S ribosomal subunits [1]. In case of successful purification of subunits, a single peak is present in the sample when monitoring the absorbance at 260 nanometers [3].

4.2.1. LAB MEDIA: Figure 2B. *Video editor: Highlight the peak labeled "40S" and "60S".*

4.2.2. LAB MEDIA: Figure 3 *Video editor: Highlight the peaks in A - B*

4.3. Under 10 millimolar magnesium acetate conditions, wild-type 40S and 60S subunits reassociated to form a single 80S ribosomal particle peak [1]. The peak of free 40S subunits was visible because 40S subunits were used in a molar excess compared to 60S subunits [2].

4.3.1. LAB MEDIA: Figure 4A. *Video editor: Highlight the tall peak labeled "80S"*

4.3.2. LAB MEDIA: Figure 4A. *Video editor: Highlight the peak labeled "40S"*

4.4. When tRNA was added to the 10 millimolar magnesium acetate condition, an additional peak appeared at the top of the gradient, corresponding to excess tRNA [1].

4.4.1. LAB MEDIA: Figure 4B. *Video editor: Highlight the small peak at the top of the gradient, near the 10% sucrose end.*

4.5. Increasing the magnesium acetate concentration to 20 millimolar led to the formation of narrower peaks in wild-type reassociation assays [1].

4.5.1. LAB MEDIA: Figure 4C. *Video editor: Highlight the sharp and narrow peak labeled "80S".*

Pronunciation Guide:

Reassociation

Pronunciation link: <https://www.merriam-webster.com/dictionary/reassociation>

IPA: /ˌriːəˌsoʊsiˈeɪʃən/

Phonetic Spelling: ree-uh-soh-see-ay-shuhn

Assay

Pronunciation link: <https://www.merriam-webster.com/dictionary/assay>

IPA: /ˈæˌseɪ/

Phonetic Spelling: a-say

Ribosomal

Pronunciation link: <https://www.merriam-webster.com/dictionary/ribosomal>

IPA: /raɪˈboʊsəmə/

Phonetic Spelling: rye-boh-suh-muhl

Subunits

Pronunciation link: <https://www.merriam-webster.com/dictionary/subunit>

IPA: /ˈsʌbˌjuːnɪt/

Phonetic Spelling: sub-yoo-nit

Eukaryotic

Pronunciation link: <https://www.merriam-webster.com/dictionary/eukaryotic>

IPA: /ˌjuːˈkæriˈɑːtɪk/

Phonetic Spelling: yoo-ka-ree-aa-tik

Sucrose

Pronunciation link: <https://www.merriam-webster.com/dictionary/sucrose>

IPA: /ˈsuːˌkroʊs/

Phonetic Spelling: soo-krohs

Polypropylene

Pronunciation link: <https://www.merriam-webster.com/dictionary/polypropylene>

IPA: /ˌpɑːliˈproʊpəˌliːn/

Phonetic Spelling: pah-lee-proh-puh-leen

Centrifuge

Pronunciation link: <https://www.merriam-webster.com/dictionary/centrifuge>

IPA: /ˈsentɹəˌfjuːdʒ/

Phonetic Spelling: sen-truh-fyooj

Ultracentrifuge

Pronunciation link: <https://www.merriam-webster.com/dictionary/ultracentrifuge>

IPA: /ˌʌltrəˈsentɹəˌfjuːdʒ/

Phonetic Spelling: ul-truh-sen-truh-fyooj

Peristaltic

Pronunciation link: <https://www.merriam-webster.com/dictionary/peristaltic>

IPA: /ˌperəˈstɔːltɪk/

Phonetic Spelling: peh-ruh-staal-tik

Spectrophotometer

Pronunciation link: <https://www.merriam-webster.com/dictionary/spectrophotometer>

IPA: /ˌspektrəfoʊˈtɑːmɛtər/

Phonetic Spelling: spek·truh·foh·taa·muh·ter

Capillary

Pronunciation link: <https://www.merriam-webster.com/dictionary/capillary>

IPA: /ˈkæpəˌlɛri/

Phonetic Spelling: kap·uh·leh·ree

Lysate

Pronunciation link: <https://www.merriam-webster.com/dictionary/lysate>

IPA: /ˈlaɪˌseɪt/

Phonetic Spelling: lye·sayt

Fractionator

Pronunciation link: No confirmed link found

IPA: /ˈfrækʃəˌneɪtər/

Phonetic Spelling: frak·shuh·nay·ter

Dissociation

Pronunciation link: <https://www.merriam-webster.com/dictionary/dissociation>

IPA: /dɪˌsoʊsiˈeɪʃən/

Phonetic Spelling: dih·soh·see·ay·shuhn

Magnesium

Pronunciation link: <https://www.merriam-webster.com/dictionary/magnesium>

IPA: /mæɡˈniːziəm/

Phonetic Spelling: mag·nee·zee·uhm

Acetate

Pronunciation link: <https://www.merriam-webster.com/dictionary/acetate>

IPA: /ˈæsəˌteɪt/

Phonetic Spelling: a·suh·tayt

Molecular

Pronunciation link: <https://www.merriam-webster.com/dictionary/molecular>

IPA: /məˈlekjələr/

Phonetic Spelling: muh·lek·yuh·ler

Nucleic

Pronunciation link: <https://www.merriam-webster.com/dictionary/nucleic>

IPA: /nuːˈkliːɪk/

Phonetic Spelling: noo·klee·ik